SUBJECT: **Taxonomic Identification Report for R-21-0001**

FROM: Md Mizanur Rahman, Ph.D.
Risk Assessment Branch 2
New Chemicals Division

TO: Dr. Gwen McClung
Technical Integrator
Risk Assessment Branch 2
New Chemicals Division

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********** [redacted] **********

I. TAXONOMY AND CHARACTERIZATION

A. Parent and Recipient Microorganisms

EPA has received a TSCA Experimental Release Application (TERA) from [redacted] for [redacted] intergeneric subject microorganisms, designated as [redacted] and [redacted]. The subject strains are genetically engineered to evaluate the ability to affect nitrogen fixation and enhance nitrogen acquisition in corn plants.

The recipient microorganism for this TERA is [redacted] which was developed from the parental strain [redacted]. The submitter obtained the parental [redacted] strain from the [redacted] with the deposition number [redacted].The strain is also deposited in the [redacted]) as [redacted], the [redacted]) as [redacted], and the [redacted] as [redacted].

To create subject strains, intra- and intergeneric genes were introduced into the recipient strain using [redacted]. All [redacted] subject strains harbor [redacted] from [redacted], and [redacted] and [redacted] genes from [redacted]. A number of genes from [redacted] and [redacted] were integrated into the genomes of the respective subject strains. However, with the exception of synthetic [redacted] gene based on the sequence from [redacted] which was integrated into strain [redacted], these genes are considered to be intergeneric rather than intrageneric due to DNA modifications to [redacted] [redacted] used during the [redacted] process. These include: [redacted] and [redacted] There were no DNA modifications for [redacted] so it is intrageneric. Not all subject strains contain identical intergeneric genes. The subject strains all have [redacted] and [redacted] [redacted] from [redacted]. As per the TERA, all genes are chromosomally integrated into the respective subject strains which were verified

by whole genome sequencing. was used during plasmid construction , however, no subject strain contain the gene.

[redacted] ( ).

[redacted]). A more accurate representation of phylogenetic relationships among these bacteria was attained in sequences were determined for). Phylogenetic analyses showed that [redacted]

Modern taxonomy is primarily based on the phylogenetic analysis of the 16S rRNA gene . However, comparative genomics based phyligeny is becoming a popular tool to determine the taxonomy of microorganisms. conducted a comparative genome analysis using the genomes of strains of genomes. The study demonstrates that species cluster into and revealed metabolic diversity within the subgroups. The study also suggested that the major reported [redacted]

[redacted] based on phenotypic characteristics and 16s ribosomal sequence phylogeny. According to the authors, cells of the [redacted]. The morphological features of the subject microorganisms are expected to be similar to the recipient organism. The subject organisms can be uniquely identified and differentiated from the recipient organism by and detection of construct insertion into the genome by whole genome sequencing. In addition, all strains are expected to be improved for nitrogen fixation compared to the recipient strain.

The strain [redacted] was validated by [redacted] as [redacted] through whole genome sequencing with three biological replicates independently grown in three liquid cultures. Sequences of the three replicates are [redacted] to each other and showed [redacted] identity to the [redacted] genome deposited with [redacted] as [redacted]. [redacted] is the only example of a publicly available [redacted] genome sequence. Whole-genome [redacted] selected representative [redacted] genomes along with those belonging to [redacted] and [redacted] were calculated using [redacted] was used as the out-group clade. All [redacted] assemblies cluster together within a clade (see TERA, attachment 3) including [redacted] strain (accession [redacted]). The [redacted] strains form a close, but separate cluster. [redacted] values were also calculated between [redacted] assemblies and representative [redacted] and [redacted] from [redacted]. The average [redacted] compared to [redacted]; in contrast, it was [redacted]. The taxonomy of the subject microorganisms was also verified through [redacted] belonging to [redacted]

Taxonomy of the Recipient Microorganism

Domain:	Bacteria
Phylum:	Firmicutes
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Conclusion regarding the recipient strain: Based on the [redacted] analysis, [redacted] and [redacted] values, the designations of the parent, recipient strain and the subject strains as [redacted] are acceptable.

B. Donor Microorganisms

The intergeneric [redacted] *and* [redacted] genes introduced into the subject strains are derived from [redacted]. [redacted] is well known for its ability to cause life-threatening infections. This bacterium can thrive as a commensal on and in human tissues without causing much problems. Recent findings on [redacted] carriage on skin, mucosa, and in wounds indicate the presence of large numbers of [redacted], yet its abundance can be without major implications for the host [redacted]).

[redacted]) introduced into the subject strains are based on the nucleotide sequence from [redacted], known as [redacted], is a [redacted]ck having significant effects on production and animal welfare. This organism is distributed throughout Europe, Africa, Asia, and the Americas [redacted]).

A number of intergeneric genes, derived from [redacted] and [redacted], were integrated into genomes of respective subject strains. [redacted], [redacted]).

The subject strains all have [redacted] and [redacted] from [redacted] strain [redacted] is a well-researched strain [redacted] is a common [redacted] that is ubiquitous in the environment. It is also used industrially for enzyme production.

References

[redacted]

[redacted]